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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/725,309	11/29/2000	Alok Singh	79,212	8594

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Associate Counsel (Patents), Code 1008.2
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EXAMINER
HUTSON, RICHARD G

ART UNIT	PAPER NUMBER
1652	

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Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No. 09/725,309	Applicant(s) SINGH ET AL.	
	Examiner Richard G Hutson	Art Unit 1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *29 March 2002* .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-14* is/are pending in the application.

4a) Of the above claim(s) *1 and 2* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *3-14* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s) *2* .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

DETAILED ACTION

Claims 1-14 are at issue and are present for examination.

Election/Restrictions

Applicant's election with traverse of Group II, Claims 3-14 in Paper No. 6 is acknowledged. The traversal is on the ground(s) that Group I has been classified in class 435, 188 and Group II, which has been classified in class 435, subclass 440 should have properly been classified in class 435, subclass 188, and thus the search and examination of the entire application could be made without serious burden and restriction is improper. Applicants traversal is not persuasive, because while the proper classification of Group II in class 435, subclass 440 or 188 could be argued, clearly both of these subclasses would need to be searched to properly search those claims in Group II, while only class 435, subclass 188 would need to be searched for Group I. Further class 435, subclass 183, as well as class 530, subclass 350 would need to be searched to properly search Group I, thus the search and examination of both groups I and II while overlapping is not coextensive, thus the search and examination of both groups would cause a serious burden.

The requirement is still deemed proper and is therefore made FINAL.

Claims 1 and 2 are withdrawn from further consideration by the examiner, 37 CFR 1.142(b), as being drawn to a non-elected invention, the requirement having been traversed in Paper No. 6.

Information Disclosure Statement

The listing of references in the specification is not a proper information disclosure statement. 37 CFR 1.98(b) requires a list of all patents, publications, or other information submitted for consideration by the Office, and MPEP § 609 A(1) states, "the list may not be incorporated into the specification but must be submitted in a separate paper." Therefore, unless the references have been initialed on form PTO-1449 or cited by the examiner on form PTO-892, they have not been considered.

Applicants filing of information disclosures, Paper No. 2, filed 11/29/2000, and is acknowledged. Those references considered have been initialed.

Specification

The disclosure is objected to because of the following informalities:

On page 8, lines 15 and 16, the specification refers to (Cho and Corona (1993) Journal of Biological Chemistry 26:9238-9245). The actual volume of the referred to reference is **268**, not **26**.

The abstract of the invention, second sentence, recites "A a stable carrier..." This should be "A stable carrier..." Further this sentence does not end with a period.

Appropriate correction is required.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 3-14 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 9 (10 and 12-14 dependent on) is indefinite in that it is confusing in the recitation "... stabilizing enzymes **comparing**:." For the purpose of compact prosecution, this recitation is interpreted as "... stabilizing enzymes **comprising**:."

Claim 11 recites the limitation "the carrier" of claim 7. There is insufficient antecedent basis for this limitation in the claim. Did applicants intend this claim to depend from claim 9? This is presumed for the purpose advancing prosecution.

Claim 12 recites the limitation "the metal oxide particles" of claim 9. There is insufficient antecedent basis for this limitation in the claim. Did applicants intend this claim to depend from claim 11? This is presumed for the purpose advancing prosecution.

Claims 3 (4-8 dependent on) and 9 (10-14 dependent on) are indefinite in the recitation "...genetically engineering an enzyme to include a stabilizing amino acid substitution..." is unclear. Specifically it is unclear in applicants reference to a stabilizing amino acid substitution, since applicants have not taught any such stabilizing amino acid substitutions. Applicants specification has merely taught the addition of a polyhistidine sequence of amino acids to the amino terminus of a known enzyme. Since this would not be considered by one of skill in the art to be a "stabilizing amino acid **substitution"**, applicants claim to such is unclear. For the purpose of advancing

prosecution, this recitation is interpreted as meaning "any amino acid substitution, or addition" that is used attach a protein to a salt group.

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 3-14 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Claims 3-14 are directed to all possible methods for stabilizing an enzyme comprising genetically engineering an enzyme to include a stabilizing amino acid substitution and binding the genetically engineered enzyme to a salt on the outer surface of a vesicle (claims 3-8) or particular inorganic carrier (claims 9-14). The specification, however, only provides a single method of genetically engineering an enzyme and the disclosed method only encompasses the addition of histidine residues on the amino terminus of the *E. coli* enzyme thioesterase I. There is no disclosure of any method of genetically engineering an enzyme to include a "stabilizing amino acid substitution" (See above 112 2nd paragraph rejection). There is no disclosure of any particular structure to function/activity relationship or any "stabilizing amino acid substitution" for any enzyme. Given this lack of additional representative species as encompassed by the claims, Applicants have failed to sufficiently describe the claimed

invention, in such full, clear, concise, and exact terms that a skilled artisan would recognize Applicants were in possession of the claimed invention.

Applicant is referred to the revised guidelines concerning compliance with the written description requirement of U.S.C. 112, first paragraph, published in the Official Gazette and also available at www.uspto.gov.

Claims 3-14 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method for stabilizing an enzyme comprising genetically engineering an enzyme to include a polyhistidine sequence on the amino or carboxyl terminus, does not reasonably provide enablement for any method for stabilizing an enzyme comprising genetically engineering an enzyme to include any stabilizing amino acid substitution. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

Factors to be considered in determining whether undue experimentation is required, are summarized in In re Wands (858 F.2d 731, 8 USPQ 2nd 1400 (Fed. Cir. 1988)) as follows: (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claim(s).

Claims 3-14 are so broad as to encompass all possible methods for stabilizing an enzyme comprising genetically engineering an enzyme to include a stabilizing amino acid substitution and binding the genetically engineered enzyme to a salt on the outer surface of a vesicle (claims 3-8) or particular inorganic carrier (claims 9-14). The scope of the claims is not commensurate with the enablement provided by the disclosure with regard to the extremely large number of methods of genetically engineering any stabilizing amino acid substitution of any enzyme. Since the amino acid sequence of a protein determines its structural and functional properties, predictability of which changes can be tolerated in a protein's amino acid sequence and obtain the desired activity requires a knowledge of and guidance with regard to which amino acids in the protein's sequence, if any, are tolerant of modification and which are conserved (i.e. expectedly intolerant to modification), and detailed knowledge of the ways in which the proteins' structure relates to its function. However, in this case the disclosure is limited to the addition of six histidine residues on the amino-terminus of *E. coli* thioesterase.

While recombinant and mutagenesis techniques are known, it is not routine in the art to screen for multiple substitutions or multiple modifications, as encompassed by the instant claims, and the positions within a protein's sequence where amino acid modifications can be made with a reasonable expectation of success in obtaining the desired activity/utility are limited in any protein and the result of such modifications is unpredictable. In addition, one skilled in the art would expect any tolerance to modification for a given protein to diminish with each further and additional modification, e.g. multiple substitutions.

The specification does not support the broad scope of the claims which encompass all methods for genetically engineering any stabilizing amino acid substitution into an enzyme structure because the specification does not establish: (A) those enzymes or regions of those enzymes' structure which may be modified effecting stability without effecting activity; (B) the general tolerance of said enzyme to modification and extent of such tolerance; (C) a rational and predictable scheme for modifying any amino acid residue of said enzyme with an expectation of obtaining the desired biological function; and (D) the specification provides insufficient guidance as to which of the essentially infinite possible choices is likely to be successful. Because of this lack of guidance, the extended experimentation that would be required to determine which substitutions would be acceptable to effect the enzyme stabilization claimed and the fact that the relationship between the sequence of a peptide and its tertiary structure (i.e. its activity and stabilization) are not well understood and are not predictable (e.g., see Ngo et al. in The Protein Folding Problem and Tertiary Structure Prediction, 1994, Merz et al. (ed.), Birkhauser, Boston, MA, pp. 433 and 492-495, Ref: U, Form-892), it would require undue experimentation for one skilled in the art to arrive at the majority of those methods of the claimed genus having the claimed means of enzyme stabilization.

Thus, applicants have not provided sufficient guidance to enable one of ordinary skill in the art to make and use the claimed invention in a manner reasonably correlated with the scope of the claims broadly including any method for stabilizing an enzyme comprising genetically engineering an enzyme to include a stabilizing amino acid substitution. The scope of the claims must bear a reasonable correlation with the scope

of enablement (In re Fisher, 166 USPQ 19 24 (CCPA 1970)). Without sufficient guidance, the claimed methods are unpredictable and the experimentation left to those skilled in the art is unnecessarily, and improperly, extensive and undue. See In re Wands 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir, 1988).

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 9-12 and 14 are rejected under 35 U.S.C. 102(b) as being anticipated by Qiagen Product Guide, 1997, pages 106-110.

Qiagen Product Guide teaches the QIAexpress Ni-NTA (nickel-nitrilotriacetic acid) protein purification system and methodsfor using this system comprising genetically engineering the insertion of a cDNA sequence encoding a desired protein (to be purified) sequence into an expression vector pQE, such that it inserts a 6X histidine tag into the protein and then adding said 6X His-tagged protein to a Ni-NTA spin column where the 6X His-tagged protein attaches to a Ni-NTA silica base material that is used to purify the 6X His-tagged protein. Thus the method taught by the Qiagen Catalog anticipates a claim to a method comprising genetically engineering an enzyme to include a stabilizing amino acid substitution (See above 112 2nd paragraph discussion);

attaching said stabilized enzyme to a metal salt of nitrilotriacetic acid on the surface of a particular inorganic (silica) carrier (claims 9-12 and 14).

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claim 13 is rejected under 35 U.S.C. 103(a) as being unpatentable over Qiagen Product Guide, 1997, pages 106-110, as applied to claims 9-12 and 14 above, and further in view of Lu et al. (Journal of Biological Chemistry, Vol 271, No. 9, pages 5059-5065, March 1996).

As discussed above, Qiagen Product Guide (1997) teaches the QIAexpress method comprising genetically engineering a 6X histidine tag into a protein and then adding said 6X His-tagged protein to a Ni-NTA spin column where the 6X His-tagged protein attaches to a Ni-NTA silica base material that is used to purify the 6X His-tagged protein.

Lu et al. teaches similar methods, as those taught by the Qiagen catalog, of protein purification of *E. coli* thioredoxins, with the following exceptions. Lu et al. rather then add a 6X His sequence to the amino or carboxyl terminus of the protein, mutate selective surface exposed residues to histidine and in addition to nickel-nitrilotriacetic

acid (NTA) salts, Lu et al. use copper- and nickel-iminodiacetic acid (IDA) salts. Lu et al. teach that both the IDA and NTA salts resulted in identical protein affinity results (page 5061, top of column 2).

Thus one of ordinary skill in the art would have been motivated to use either IDA or NTA salts in the methods taught by the Qiagen Product Guide because each of the different salts works equally well. The reasonable expectation of success comes from the results of the Qiagen Product Guide who successfully teach the purification of mouse DHFR with NTA silica and those of Lu et al. who teach the successful purification of *E.coli* thioredoxin with either IDA or NTA resins.

Claims 3-5, 7 and 8 are rejected under 35 U.S.C. 103(a) as being unpatentable over Singh (U.S. Patent Number. 5,663,387), LeJeune et al. (Biotechnology and Bioengineering Vol 54, No. 2, pages 105-114, April 1997) and Polayes et al. (Life Technologies-FOCUS, Vol 16, page 81-84. July 1994).

Singh teaches that polymerizable phospholipids have been used in the stabilization of molecular assemblies and in the development of strategies to expand the usefulness of the lipid assemblies. Singh teaches that it is desirable to provide a means for transporting enzymes and proteins that are immobilized by non-covalent binding and specifically teach methods of polymerizing an amphiphile containing a iminodiacetic acid with other polymerizable amphiphiles, forming vesicles and binding an enzyme, carbonic anhydrase, to the salt on the outer surface of the vesicles. As an example of an enzyme which could be bound to the outer surface of the taught vesicles, Singh

teach the use of an enzyme which contains several exposed histidine residues, for example, carbonic anhydrase II, since it contains six histidine residues, four of them available within a distance of 6A. Singh teach that the binding of carbonic anhydrase occurs via the non-covalent binding or chelating of the Cu^{+2} ion on the surface of the liposomes and that non-covalent binding is preferable to covalent binding because covalent enzyme binding may lead to an alteration or decrease in the enzymes activity.

LeJeune et al. teach that protein immobilization is a common method of enhancing enzyme stability and in particular LeJeune et al. teach the immobilization of phosphotriesterase, a nerve-agent hydrolyzing enzyme, using Hypolpolyurethane prepolymers via covalent binding between the enzyme and the immobilizing Hypolpolyurethane prepolymer.

Polayes et al. teach methods of genetically engineering the incorporation of a polyhistidine sequence at either the amino or carboxyl terminus of a protein for use in purifying the expressed protein. Polayes et al. teach that this 6 histidine sequence has a strong affinity for the Ni^{2+}-nitrilo-triacetic acid resin.

One of ordinary skill in the art at the time of filing would have been motivated to immobilize a nerve agent hydrolyzing enzyme, such as phosphotriesterase, as a method of enhancing the stability of the enzyme as taught by LeJeune et al. One would have been further motivated to such methods involving the immobilization of the enzyme to a liposome vesicle, wherein said vesicle comprises an amphiphile containing a iminodiacetic acid with other polymerizable amphiphiles, using non-covalent binding as taught by Singh et al. so that undesirable effects such as alterations or decreases in

enzyme activity as a result of covalent binding are avoided. One would have been further motivated to genetically engineer the specific enzyme to be immobilized, such that it comprised a string of exposed histidine residues at either the amino or carboxyl terminus of the protein that could be used in an analogous manner as used by Singh with the internal histidine residues in carbonic anhydrase II to attach the enzyme to the salt on the exterior of the vesicle taught by Singh. The reasonable expectation of success comes from the high degree of knowledge in the field as demonstrated by the results of Singh et al. who successfully attached enzymatically active carbonic anhydrase II via this mechanism and exposed histidine residues internal to the enzyme, to the outside of liposome vesicles as well as those of Polayes et al. who teach similar means of binding a genetically engineered enzyme using a heterologous polyhistidine sequence and the Nickel ion.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Richard G Hutson whose telephone number is (703) 308-0066. The examiner can normally be reached on 7:30 am to 4:00 pm, M-F.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ponnathapu Achutamurthy can be reached on (703) 308-3804. The fax phone numbers for the organization where this application or proceeding is assigned are (703) 305-3014 for regular communications and (703) 305-3014 for After Final communications.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is (703) 308-0196.

Richard Hutson, Ph.D.
Patent Examiner
Art Unit 1652
June 17, 2002